

Original Article



Efficacy of *Withania somnifera* Extract With and or Without Vitamin E on Testicular Dimensions and Semen Quality of Awassi Rams

Hanan Waleed Kasim Agwaan*

Department of Animal Production, College of Agriculture and Forestry, University of Mosul, Mosul, Iraq.



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ABSTRACT

Background: The structural health of the testes and semen quality in rams depend on their ability to withstand oxidative damage and nutritional imbalances. Although research has shown that *Withania somnifera* and vitamin E provide benefits, their specific effects on male reproductive health and their ability to reduce oxidative stress have not been determined.

Objectives: This research examined the effects of an aqueous extract of *W. somnifera* administered with vitamin E supplements on testicular dimensions and semen characteristics in rams.

Methods: The research included 24 clinically healthy rams that were ten months old and weighed 35 ± 0.5 kg. The animals were divided into four groups of six each. The T1 animals received only the basal ration, the T2 animals received *W. somnifera* at 150 mg/kg body weight, and the T3 animals received vitamin E at 100 mg/kg body weight. The T4 animals received both agents simultaneously. The study lasted 12 weeks, during which the animals received their assigned treatments orally. The researchers measured testicular dimensions twice a week and collected semen samples to analyze volume, concentration, sperm motility, and morphology.

Results: The T4 group, which received both supplements, demonstrated substantial improvements in sperm motility, ejaculate concentration, and testicular size ($P \leq 0.05$). The combined application of antioxidant and phytochemical compounds produced enhanced results, suggesting that these compounds interact with biological systems at the cellular or physiological levels.

Conclusion: The combination of *W. somnifera* and vitamin E resulted in major improvements in testicular structure and semen quality, showing promise for enhancing reproduction in rams. The research shows that using botanical antioxidants together with micronutrient supplements is an effective method for improving fertility and reproductive outcomes in livestock.

Keywords: Awassi rams, Semen quality, Vitamin E, *Withania somnifera*

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* Corresponding Author:

Hanan Waleed Kasim Agwaan, Associate Professor.

Address: Department of Animal Production, College of Agriculture and Forestry, University of Mosul, Mosul, Iraq.

E-mail: hanan_agwaan@uomosul.edu.iq



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Introduction

Animal production industries, especially sheep and goat production, face many problems, such as decreasing production and reproduction. Improving the male reproductive system is very important, and many practitioners have considered this issue in this regard (Agwaan, 2023; Chan 2024; Agwaan, 2023; Chan, 2024). The medicinal plant *Withania somnifera* is known by multiple names, including Ashwagandha, Indian ginseng, winter cherry, and horse smell. It belongs to the Solanaceae family and has traditional value in Iran and India (Mishra, 2000). Research indicates that *W. somnifera* (Ashwagandha) provides various advantages for male reproductive health beyond its established use in treating sexual problems (Welch et al., 2023). *W. somnifera* increases sexual desire and fertility when taken alone or with other medications (Mahdi, 2011). *W. somnifera* protects sperm cells through its antioxidant compounds because these compounds eliminate oxidative damage, which causes male infertility. The roots of the plant contain various bioactive compounds, including withanolides, alkaloids, and flavonoids, which have pharmacological effects (Ganze ra et al., 2003; (Mishra et al., 2000).

The herb contains anti-inflammatory compounds that are reported to help improve sperm structure and semen quality, thereby providing reproductive benefits (Mittal et al., 2025).

W. somnifera is commonly used as an herbal treatment for infertility and sexual health issues because it contains more than 80 phytochemicals, including phenolics, alkaloids, saponins, tannins, glycosides, carbohydrates, flavonoids, starch, amino acids, and terpenoids (Arya & Chauhan, 2019) that regulate reproductive hormones, such as testosterone, luteinizing hormone (LH), and follicle-stimulating hormone (FSH). By modulating these hormones levels, *W. somnifera* protects sperm cells from damage and improves their quality (Azgomi et al., 2018). Vitamin E serves as the main antioxidant that protects sperm cells from oxidative damage and helps address fertility issues (Yousif et al., 2003). Vitamin E is significant for reproduction in farm animals. Vitamin E improves sperm motility in bulls (Ratnani et al., 2017). The mechanism by which vitamin E produces these effects involves inhibiting the damaging effects of free radicals generated by lipid peroxidation (LPO) by converting a single vitamin E molecule into two stable radicals (Hajibabaei, 2016). Vitamin E and similar lipid-soluble antioxidants have been found to have favor-

able effects on the reproductive performance of boars (Al-Fathi et al., 2019; Luo et al., 2004). Reactive oxygen species (ROS) are required by spermatozoa to carry out several important processes, including the acrosome reaction, sperm penetration, and sperm fusion (Khan, 2011). However, excessive amounts of ROS can damage sperm membranes through LPO (Khan, 2011; Khan, 2012). Vitamin E plays a critical function in maintaining cellular integrity and counteracting oxidative processes that compromise reproductive efficiency.

In addition, *W. somnifera* reduces oxidative stress in sperm by inhibiting the production of hydroperoxides, which can be detrimental to the sensitive lipids in sperm membranes (Chow, 2001).

Vitamin E prevents oxidative damage in spermatogenic cells, Sertoli cells, and Leydig cells (Malmir et al., 2021). A deficiency of vitamin E in testicular tissues causes oxidative stress, thereby lowering testosterone production, because vitamin E acts as an antioxidant that protects Leydig cells from damage. Vitamin E is a potent antioxidant that protects the cell membranes of Leydig cells from LPO by neutralizing free radicals (Hutabarat et al., 2023).

Although several studies have examined the separate influences of *W. somnifera* and vitamin E on various aspects of reproductive function, a gap remains in understanding how they work together, especially in rams. This research investigated whether the combined effects of an aqueous extract of *W. somnifera* and vitamin E supplementation can increase testicular size by improving testicular cellular health, enhancing hormonal balance, and promoting spermatogenic activity; improving semen quality; and ultimately enhancing the reproductive performance of genetically superior Awassi rams by providing them with healthy, motile sperm capable of traveling to the egg for fertilization. These factors are determined by the quantity and quality of the semen produced by the ram (Santolaria et al., 2023). The purpose of this work was to determine how a mixture of vitamin E and *W. somnifera* affects testicular parameters and semen-quality characteristics in Awassi rams. The proposed mechanisms underlying the reproductive effects of *W. somnifera* are summarized in Figure 1.

Materials and Methods

The study was conducted at a local field and included 24 clinically healthy rams aged 10 to 12 months and weighing 35 ± 0.5 kg. The study lasted three months (from the beginning of May to the end of August 2025). The 24 animals were randomly distributed into four equally

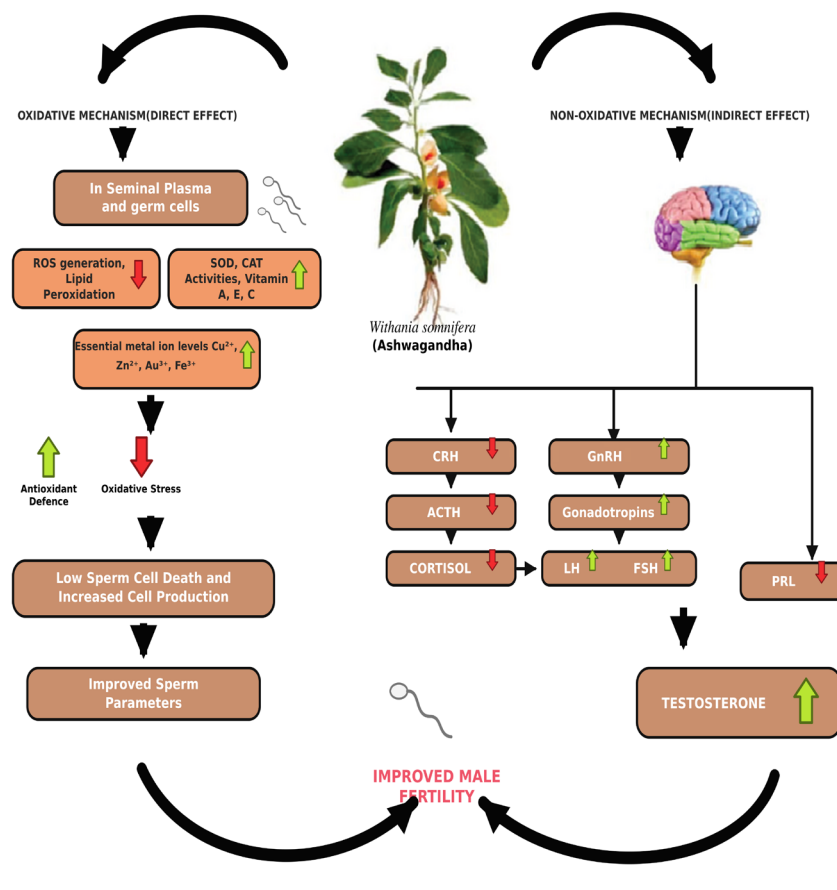


Figure 1. Mechanistic pathways through which *W. somnifera* modulates sperm quality and regulates the reproductive hormonal profile (Mittal et al., 2025)

sized groups (n=6 each). The T1 animals received only the basic ration. The T2 animals received *W. somnifera* at a dose of 150 mg/kg body weight, and the T3 animals received vitamin E at a dose of 100 mg/kg body weight. The T4 animals received both vitamin E and *W. somnifera*. The research period lasted 12 weeks.

Preparation of the aqueous Ashwagandha extract

W. somnifera roots were cut 2-cm-long pieces to facilitate drying. After being dried in the shade, the roots were ground into a coarse powder. Soxhlet extraction was performed with 95% ethanol for 72 hours at 70 to 80 degrees Celsius. After 72 hours, the extract was concentrated, and the solvent was removed using a rotary evaporator. The extract was stored at 4 °C until needed. The prepared extract was diluted in distilled water and administered orally to each ram.

Measurement of testicular dimensions

We measured testicle length and width by pressing the testicles against the scrotum, and we measured scrotal

thickness using a caliper. To determine scrotal circumference, we used a tape measure to assess the testes at their point of greatest width (Ahmad et al., 2011).

Semen collection and analysis

Semen was collected from the rams, which had been trained to use an artificial vagina, and was evaluated immediately after collection for its general characteristics, including semen volume, concentration, mass motility, and the numbers of live, dead, and morphologically abnormal spermatozoa. Semen volume (L) was determined using a graduated collection tube with a minimum reading accuracy of 0.1 mL. To evaluate mass motility, a 10 µL aliquot of undiluted semen was placed at two separate points on a pre-warmed microscope slide positioned on a stage maintained at 37 °C, and activity was scored on a scale ranging from 0 to 5. Semen volume, mass motility, and individual motility (0–100%) were estimated (Chemineau et al., 1991). Sperm concentration was determined using a spectrophotometer (Salisbury et al., 1961). The percentages of abnormal, live, and dead sperm were calculated

(Chemineau et al., 1991). A commercial enzymatic immunoassay (ELISA) kit (DiaPlus Inc., USA) was used to measure serum testosterone concentrations (Alkhabab et al., 2021).

Statistical analysis

Data processing was conducted using the statistical analysis system (SAS, 2007), and variations among group means were assessed through Duncan's multiple range test (Duncan, 1955). The analysis of changes in body weight, as well as testicular and semen parameters, was performed using a specific statistical model.

$$Y_{ij} = \mu + T_i + e_{ij}$$

y_{ij} = the individual observation

μ = the overall mean of the trait under investigation,

T_i = the influence of the applied treatment or experimental group

e_{ij} = the residual experimental error

Results

The findings presented in Table 1 regarding the chemical composition of *W. somnifera* root show that it contains fat, fiber, protein, carbohydrates, ash, and moisture.

The results in Table 2 regarding testicular measurements revealed a significant increase ($P \leq 0.05$) in the experimental groups (T2, T3, and T4) compared to the control group (T1). The testicular size measurements show that testicular development and operational performance.

The findings presented in Table 3 demonstrate that all treated groups exhibited marked enhancements in semen traits compared with the control group. Furthermore, the rise in testosterone concentrations shown in Table 3 was significantly greater ($P \leq 0.05$) in the treated groups, highlighting a clear endocrine response to supplementation. The results regarding the testicular parameters in Table 3 showed the positive impact of vitamin E on semen characteristics. Vitamin E treatment led to a major and statistically proven improvement in sperm motility and concentration in rams, resulting in better semen quality.

Discussion

The increase in final body weight may be attributed to the ability of *W. somnifera* to increase testosterone levels, which promote muscle growth (Taqa & Idrees, 2023). It may also be attributed to the role of vitamin E as an important antioxidant that enhances immune function and overall health, which can positively affect growth performance and body weight and increase rumen microbial growth (Kasim et al., 2023). This outcome is consistent with another research (Taqa & Idrees, 2023), reporting that administering (200 mg/kg) of Ashwagandha root extract to rats increased muscle growth. This effect was attributed to two causes: (i) an increase in testosterone, which encourages muscular development; and (ii) by reducing circulating cortisol, a well-known catabolic mediator, Ashwagandha appears to enhance mitochondrial energy production and functional efficiency while simultaneously diminishing the activity of Mg^{2+} -dependent ATPase, the enzyme primarily involved in ATP hydrolysis.

The compounds in *W. somnifera* roots, together with vitamin E, produce changes in testicular development and function that lead to increased body weight and improved metabolic health in rams because they boost anabolic hormones and reduce oxidative stress, culminating in increased body weight and improved metabolic health in rams.

Table 1. Chemical composition of *W. somnifera* root

Parameter	Value
Moisture	7.2
Ash	4.2
Fat	0.9
Fiber	33.3
Protein	3.3
Carbohydrates	51.1
Caloric value	245

Table 2. Effects of *W. somnifera* and vitamin E supplementation on body weight and testicular morphometry in Awassi rams

Parameters	Control (T1)	<i>W. somnifera</i> (T2)	Vitamin E (T3)	<i>W. somnifera</i> + Vitamin E (T4)
Initial body weight	35.2±0.8 ^a	34.8±0.7 ^a	36±0.9 ^a	35.5±0.8 ^a
Final body weight	45.5±1 ^a	47.2±1.1 ^b	46.8±1.2 ^{ab}	48±1 ^b
Left testes length (cm) (before)	8.5±0.05 ^a	9.8±0.04 ^b	9.4±0.05 ^b	11.7±0.03 ^c
Left testes depth (cm) (after)	4.2±0.06 ^a	4.5±0.05 ^b	4.7±0.04 ^b	5.8±0.05 ^c
Scrotal circumference before the experiment	23.5±0.2 ^a	25±0.3 ^b	25.2±0.3 ^b	25.2±0.3 ^b
Scrotal circumference after the experiment	23.5±0.2 ^a	0.3±25.2 ^b	0.3±25.4 ^b	0.3±28 ^c
Scrotal thickness	0.3±0.1 ^a	0.4±0.2 ^b	0.5±0.2 ^b	0.1±0.6 ^c

^{a, b, ab, c} Superscripts shown in a row with different letters indicate statistically significant differences between treatment means ($P \leq 0.05$).

Note: There was a significant ($P \leq 0.05$) rise in final body weight in the T2 and T4 groups compared with the control group.

The results of this study support earlier studies showing that body weight directly affects testicular size, volume, and morphometric characteristics (Salhab et al., 2001; Ramírez-Bautista et al., 2023).

The enhancement of semen traits leads to improved reproductive system function by improving semen quality. This finding aligns with the findings of another research (Finelli et al., 2021), noting that *W. somnifera* enhances reproductive system function by enhancing semen quality and increasing enzymatic activity in seminal plasma (Mikulska et al., 2023).

The ways in which *W. somnifera* influences the reproductive system and fertility can be categorized into oxidative and non-oxidative mechanisms. The oxidative mechanism involves the modulation of antioxidant activity (Sengupta et al., 2018), along with the regulation of antioxidant enzymes and the cofactors necessary for the optimal functioning of these enzymes. Conversely, nonoxidative mechanisms primarily encompass the effects of *W. somnifera* on the hypothalamic-pituitary-gonadal (HPG) axis, in addition to its antistress effects through the hypothalamic-pituitary-adrenal (HPA) axis (Wiciński et al., 2023). These direct and indirect mechanisms and their effects on sperm quality and reproductive hormones are summarized in Figure 2.

Research on *W. somnifera* or Ashwagandha shows its ability to address sperm-related problems that can lead to fertility issues. Multiple bioactive compounds are present in the plant's roots, with withanolides representing a major group (Ganzer et al., 2003) along with several alkaloids and flavonoids (Mishra et al., 2000). Scientists

believe that the plant contains potent antioxidants, and research indicates that these compounds can address reproductive problems. Its anti-inflammatory properties may also improve sperm quality (Mittal et al., 2025).

Research shows that this herb supports sperm function during fertility treatments, including IVF and IUI procedures. Research using various extract amounts have demonstrated that *W. somnifera* enhances multiple semen parameters. *W. somnifera* extract is a popular herbal medicine for treating infertility and sexual dysfunction. By measuring LPO, research has shown that *W. somnifera* can donate electrons, thereby stopping the chain reaction caused by free-radical attacks that would otherwise increase the overall production of ROS (Gadelha et al 2015).

It can be inferred that the root extract of *W. somnifera* reduces growth due to its ability to facilitate smaller chemical reactions. Additionally, the extract leads to an increase in the size of metal ions in the body, which is beneficial because these metal ions are essential for the functioning of enzymes (Shukla et al., 2011). The results presented in Table 3 showed a significant improvement ($P < 0.05$) in sperm motility and semen concentration, which aligns with other findings (Tahvilzadeh et al., 2016; Gupta et al., 2013). These findings noted the role of *W. somnifera* roots in enhancing alanine levels in semen. Alanine plays a crucial role in protecting against oxidative stress, helping to reduce LPO and ultimately improving sperm concentration and motility. In addition, the increase in testosterone levels observed in the present study following treatment with *W. somnifera* extract enhanced sperm concentration and motility.

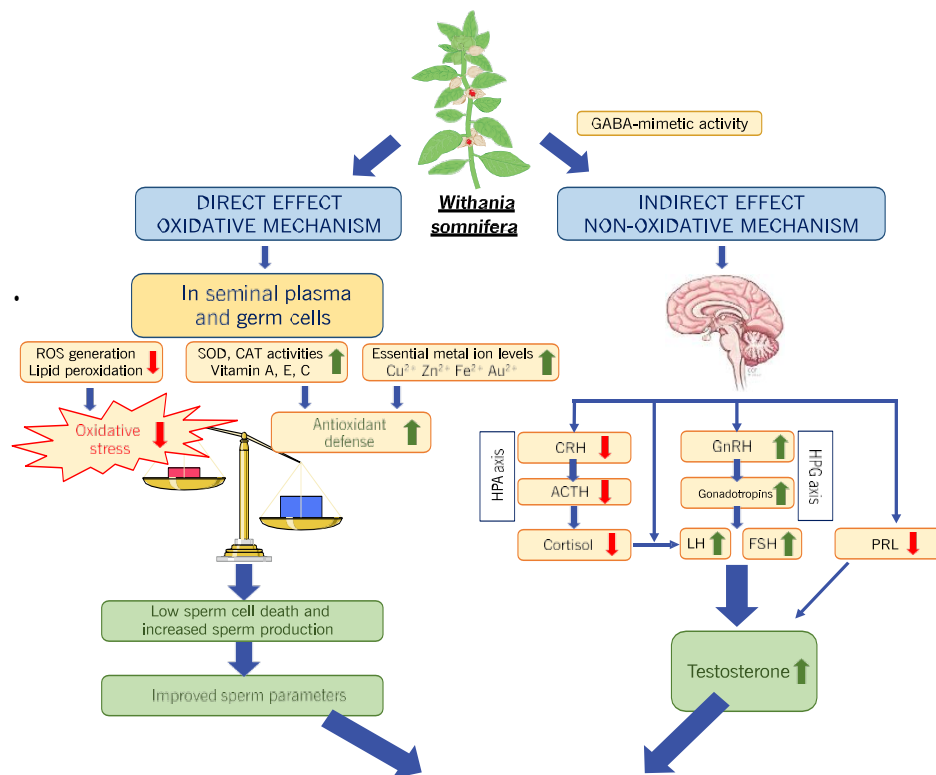


Figure 2. *W. somnifera* enhances sperm quality and reproductive hormones both directly and indirectly (Sengupta et al., 2018)

In addition to these mechanisms, the roots of *W. somnifera* are believed to contain considerable amounts of lactate and lactate dehydrogenase (LDH), components that may play a key role in stimulating the Krebs cycle and subsequently elevating intracellular cAMP and ATP levels. As suggested (Tahvilzadeh et al., 2016), these biochemical alterations may account for the observed

improvements in sperm motility and concentration following treatment with *W. somnifera* in T2 and T4 compared with the control group.

The rise in testosterone concentration (Table 3) is in agreement with another research (Sharma, 2024), reporting that supplementation with *W. somnifera* might help

Table 3. Effect of *W. somnifera* and vitamin E on semen characteristics and testosterone levels in experimental Awassi rams

Characteristics	Mean±SE			
	Control (T1)	<i>W. somnifera</i> (T2)	Vitamin E (T3)	Vitamin E + <i>W. somnifera</i> (T4)
Semen volume (mL)	0.8±0.05 ^a	1.2±0.07 ^b	1±0.06 ^{ab}	1.3±0.08 ^b
Semen concentration (×10 ⁹ /ejaculate)	2.5±0.15 ^a	3.8±0.2 ^b	3.2±0.18 ^{ab}	4±0.22 ^b
Sperm mass motility (%) (1–5)	55±2 ^a	70±1.8 ^b	65±2 ^{ab}	72±1.5 ^b
Individual sperm motility (%)	50±2.5 ^a	65±2.2 ^b	60±2.4 ^{ab}	68±2.0 ^b
Live sperm (%)	85±1.5 ^a	92±1.2 ^b	88±1.4 ^{ab}	94±1.0 ^b
Dead sperm (%)	20±1 ^a	14±0.8 ^b	16±1 ^c	12±0.7 ^d
Abnormal sperm (%)	10±0.5 ^a	8±0.4 ^b	9±0.5 ^c	6±0.3 ^d
Serum testosterone (ng/mL)	2±0.1 ^a	2.8±0.15 ^b	2.5±0.12 ^c	3.2±0.16 ^d

a, b, ab, c, d Different superscripts within a row are significantly different ($P \leq 0.05$).

balance hormones related to reproduction and improve physiological responses to sexual stimulation by elevating testosterone levels.

Moreover, numerous studies indicate that the phytochemical constituents of *W. somnifera* exert notable effects on the male reproductive system, primarily through their antioxidant and detoxifying actions and their ability to modulate key sex hormones (Tahvilzadeh et al., 2016; Kumar et al., 2015; Ambiyeh et al., 2013). These results agree with the findings of Ambiyeh et al. (2013), who observed that treatment with *W. somnifera* root extract led to increased testosterone levels.

Similarly, the data presented in Table 3 demonstrated a significant rise in the proportion of viable sperm, accompanied by a marked reduction in dead and morphologically abnormal sperm cells in the treated groups (T2 and T4) compared with the control group. These results align with another research (Bhargavan et al., 2015), reporting that oral treatment with 200 mg of *W. somnifera* significantly boosted sperm count and reduced oxidative damage, which may, in turn, contribute to a reduction in morphological abnormalities in the sperm of adult male rats. Owing to its abundant antioxidant constituents and notable anti-inflammatory actions, *W. somnifera* has been used to address subfertility by increasing sperm count, enhancing overall semen quality, and supporting the regulation of reproductive hormone profiles (Roy et al., 2025).

The positive effects on semen characteristics shown in Table 3 may result from vitamin E's role as a free-radical scavenger, which protects sperm from ROS. By preventing the oxidation and peroxidation of semen (Yue et al., 2010; Hussain et al., 2012), vitamin E functions as an antioxidant. Oxidative stress can impair sperm motility by altering membrane fluidity. The high concentration of polyunsaturated fatty acids (PUFAs) in the sperm membrane is directly associated with these changes in fluidity, making sperm more prone to LPO (Al-Asadi et al., 2025).

Vitamin E functions as a critical factor in spermatogenesis because it prevents free radical formation and protects membrane lipids from oxidative damage. It also protects sperm structure and maintains acrosomal membrane stability, thereby supporting successful fertilization. The results of this study are consistent with those reported by Kutlubay et al. (2007) who showed that vitamin E supplementation protects testicular cells from oxidative damage while improving sperm survival and reducing lipid damage. Improvement in sperm motility

and concentration following vitamin E supplementation is consistent with the findings of Yousef et al. (2003), who emphasized that vitamin E is important for improving semen quality by increasing sperm motility and sperm concentration in the ejaculate, thereby raising fertility rates in rams.

Cells involved in semen production obtain increased metabolic energy through vitamin E consumption, which leads to higher sperm numbers in seminal plasma (Yue et al., 2010; Hussain et al., 2012). The increase in testosterone levels occurs because vitamin E stimulates Leydig cells to generate more testosterone and increase secretions from the accessory reproductive organs (Soleimani et al., 2009; Mahmoud et al., 2013). The hormonal increase occurs because vitamin E protects testicular structure, thereby enabling improved Leydig cell function and enlarging the seminiferous tubules, which enhances steroid production (Hong et al., 2009). The T3 group showed a greater testicular size, according to Table 2, because vitamin E acts as an antioxidant that protects testicular tissues from oxidative stress (Bas et al., 2023).

Testicular morphometry depends on four essential parameters: weight, length, breadth, and volume, which are used to measure spermatogenic efficiency and sperm output. Vitamin E supplementation led to improved testicular morphometric measurements. Vitamin E supports Leydig and Sertoli cell function, resulting in increased androgen-binding protein and testosterone production. Vitamin E treatment also increases the size of the seminiferous tubules and the number of interstitial cells (El-Azzazi et al., 2016). Bas et al. (2023) showed that vitamin E protects testicular structures from oxidative damage, resulting in enhanced testicular dimensions.

Ethical Considerations

Compliance with ethical guidelines

This study took into consideration all relevant ethical principles.

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Conflict of interest

The author declared no conflict of interest.

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